

Amendment to the Claims:

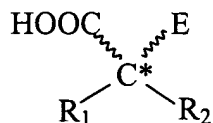
Please amend the claims as follows.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

1. (Currently amended) A method for producing an enantiomerically pure α -substituted carboxylic acid, said method comprising contacting an aldehyde or ketone with a cyanide containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing a the resulting amino nitrile or cyanohydrin intermediate with a recombinantly generated nitrilase or a polypeptide having a nitrilase activity, wherein the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure α -substituted carboxylic acid.

C²⁹
2. (Currently amended) The method according to claim 1, wherein said enantiomerically pure α -substituted carboxylic acid has the following structure:



wherein:

R₁ and R₂ are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen; or, optionally R₁ and R₂ are linked to ~~cooperate~~ to form a ~~functional~~ cyclic moiety and E is -N(R_x)₂ or -OH, wherein each R_x is -H or lower alkyl.

3. (Original) The method according to claim 2, wherein said enantiomerically pure α -substituted carboxylic acid is an α -amino acid.

4. (Original) The method according to claim 3, wherein at least one of R_1 and R_2 is substituted or unsubstituted aryl.

5. (Original) The method according to claim 4, wherein said enantiomerically pure α -amino acid is D-phenylalanine, D-phenylglycine, or L-methylphenylglycine.

6. (Original) The method according to claim 3, wherein said enantiomerically pure α -amino acid bears a substituted or unsubstituted alkyl side chain.

7. (Original) The method according to claim 6, wherein said enantiomerically pure α -amino acid is L-tert-leucine, D-alanine, or D-hydroxynorleucine.

8. (Original) The method according to claim 2, wherein said enantiomerically pure α -substituted carboxylic acid is an α -hydroxy acid.

C29
9. (Original) The method according to claim 8, wherein at least one of R_1 and R_2 is substituted or unsubstituted aryl.

10. (Presently amended) The method according to claim 8 ~~10~~, wherein said enantiomerically pure α -hydroxy acid is (S)-cyclohexylmandelic acid, mandelic acid or 2-chloro mandelic acid.

11. (Original) The method according to claim 1, wherein the cyanide is a metal cyanide or a gaseous cyanide.

12. (Original) The method according to claim 11, wherein the cyanide is an alkali cyanide.

13. (Original) The method according to claim 11, wherein the metal cyanide is sodium cyanide.

14. (Original) The method according to claim 1, wherein the ammonium salt has the formula $\text{NH}_2(\text{R})_2^+\text{X}$, wherein each R is independently -H or lower alkyl, and X is a counter ion.

C²⁹
15. (Original) The method according to claim 14, wherein X is a halide.

16. (Original) The method according to claim 15, wherein the halide is Cl^- .

17. (Original) The method according to claim 16, wherein the ammonium salt is NH_4^+Cl^- .

18. to 21. (Withdrawn)

22. (Currently amended) The method according to claim 1, wherein the nitrilase has an amino acid sequence as set forth in SEQ ID NO:2, ~~or~~ SEQ ID NO:4, or enzymatically active fragments thereof.

C²⁹
23. (Original) The method according to claim 1, wherein the nitrilase is encoded by a nucleic acid sequence as set forth in SEQ ID NO:1, ~~or~~ SEQ ID NO:3, or subsequences thereof encoding enzymatically active fragments of a nitrilase.

24. (Currently amended) The method according to claim 1, wherein the nitrilase has an amino acid sequence having at least 70% sequence identity identical to an the amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or enzymatically active fragments thereof, and has a nitrilase activity such that the nitrilase stereoselectively hydrolyzes

an amino nitrile or cyanohydrin intermediate to form the enantiomerically pure α -substituted carboxylic acid.

25. to 30. (Withdrawn)

31. (Currently amended) A method for producing an alpha-substituted carboxylic acid, the method comprising

- (a) providing an aldehyde or a ketone;
- (b) providing a cyanide-containing compound ;
- (c) providing an ammonia-containing compound or a compound comprising an ammonium salt or an amine;

(d) providing a composition comprising a recombinantly generated nitrilase or a polypeptide having a nitrilase activity;

C²⁹
(e) contacting the aldehyde or ketone of step (a) with a cyanide-containing compound of step (c) and an ammonia-containing compound or a compound comprising an ammonium salt or an amine of step (d) such that an amino nitrile or a cyanohydrin intermediate is produced; and

(f) contacting the amino nitrile or cyanohydrin intermediate of step (e) with the ~~nitrilase or polypeptide having nitrilase activity~~ composition of step (d) (e) such that the nitrilase or polypeptide hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid.

32. (Currently amended) A method for producing an alpha-substituted carboxylic acid, the method comprising

- (a) providing a composition comprising an amino nitrile or a cyanohydrin;
- (b) providing a composition comprising a recombinantly generated nitrilase or a polypeptide having a nitrilase activity; and

(c) contacting the amino nitrile or cyanohydrin ~~intermediate~~ of step (a) with the ~~nitrilase or polypeptide having nitrilase activity~~ composition of step (b) such that the nitrilase or

polypeptide having nitrilase activity hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid.

33. (Previously presented) The method of claim 31 or 32, wherein the nitrilase or polypeptide having nitrilase activity stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an enantiomerically pure alpha-substituted carboxylic acid.

34. (previously presented) The method of claim 31 or 32, wherein the alpha-substituted carboxylic acid is an alpha amino acid.

35. (previously presented) The method of claim 31, wherein the cyanide-containing compound comprises a metal or a gaseous cyanide compound.

C²⁹
36. (Currently amended) A method for producing an alpha-amino acid, the method comprising

- (a) providing an aldehyde or a ketone;
- (b) providing a cyanide-containing compound and ammonia;
- (c) providing a recombinantly generated nitrilase or a polypeptide having a nitrilase activity;
- (d) contacting the aldehyde or ketone of step (a) with the cyanide-containing compound and ammonia of step (b) such that an amino nitrile is produced; and
- (e) contacting the amino nitrile of step (d) with the nitrilase or polypeptide having nitrilase activity of step (c) such that the nitrilase or polypeptide hydrolyzes the amino nitrile to produce an alpha-substituted amino acid.

37. (previously presented) The method of claim 31, claim 32 or claim 36, wherein the reaction takes place in a single reaction vessel.

38. (NEW) The method of claim 24, wherein the nitrilase has an amino acid sequence having at least 75% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or enzymatically active fragments thereof.

39. (NEW) The method of claim 38, wherein the nitrilase has an amino acid sequence having at least 80% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or enzymatically active fragments thereof.

40. (NEW) The method of claim 39, wherein the nitrilase has an amino acid sequence having at least 85% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or enzymatically active fragments thereof.

41. (NEW) The method of claim 40, wherein the nitrilase has an amino acid sequence having at least 90% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or enzymatically active fragments thereof.

42. (NEW) The method of claim 41, wherein the nitrilase has an amino acid sequence having at least 95% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or enzymatically active fragments thereof.

43. (NEW) The method of claim 24, wherein the sequence identity is determined using a FASTA version 3.0t78 algorithm with default parameters.

44. (NEW) A method for producing an enantiomerically pure alpha-substituted carboxylic acid, said method comprising

contacting an aldehyde or ketone with a cyanide-comprising compound and an ammonia-comprising compound, an ammonium salt or an amine, and

hydrolyzing stereoselectively the resulting amino nitrile or cyanohydrin intermediate with a nitrilase, wherein the nitrilase hydrolyzes the reaction components to produce enantiomerically pure alpha-substituted carboxylic acid and has an amino acid sequence

C29
Cont

Applicant : Mark Madden et al.

Attorney's Docket No.: 09010-113001 / DIVER1440-2

Serial No. : 09/751,299

Filed : December 28, 2000

Page : 21

having at least 70% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or is encoded by a nucleic acid having at least 70% sequence identity to an amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3.
